Comm.
Dr. Jacobson
Dr. Loosli
Dr. Sommers

CHRONIC PULMONARY DISEASES

#547CR2
#547CR1-7/1/72-6/30/73
#547C -7/1/71-6/30/72
#547BR -7/1/70-6/30/71
#547B -7/1/69-6/30/70
#547A -7/1/68-6/30/69
#547R -7/1/67-6/30/68
#547 -7/1/66-6/30/67
CF265M - 11/15/60 - 11/15/64 incl.

THE COUNCIL FOR TOBACCO RESEARCH—U.S.A., INC.
110 EAST 59TH STREET
NEW YORK, N. Y. 10022
(212) 421-8565

Application For Renewal of Research Grant
(Use extra pages as needed)

First Renewal ☐ Second Renewal ☒

Date: 1/23/73

JAN 2 9 1973

JAN 29 1973

1. Principal Investigator (give title and degrees):
Joseph J. Guarneri, Ph.D., Attending Microbiologist, Director Microbiology Labs, Long Island Jewish-Hillside Medical Center/Queens Hospital Center Affiliation.

2. Institution & address:
Long Island Jewish-Hillside Medical Center
New Hyde Park, N. Y. 11040

3. Department(s) where research will be done ~~or collaboration provided~~
Department of Laboratories
Queens Hospital Center Affiliation, Long Island Jewish Hospital Center
82-68 164th St., Jamaica, N. Y. 11432

4. Short title of study.
The Influence of Extended Exposure to Cigarette Smoke on Pulmonary Resistance to Infection as Related to Alveolar Macrophage and Mucociliary Function.

5. Proposed renewal date. July 1, 1973

6. How results to date have changed earlier specific research aims: None

7. How results to date have changed earlier working hypothesis: None

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8. Any additional facilities now required? Describe briefly: None

9. Any changes in personnel? Append biographical sketches of new key professional personnel: See Page 2A

(a). No change in personnel.

(b) See page 2A for biographical sketch of new professional personnel, Marcelino F. Sierra, Ph.D.

10. Append outline of experimental protocol for ensuing year. See pages 2B - 2D

11. List publications or papers in press resulting from this or closely related work. (append reprints or manuscripts not previously sent).

J. J. Guarneri and G. A. Laurenzi. Influence of Cigarette Smoke on Pulmonary Defense Against Inhaled Bacteria: Alveolar Macrophage Numbers and Viability. ASM Abstracts page 108, 1972.

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12. Summary progress report (append in standard form as separate document, unless recently submitted). Progress Report appended.

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| Name | Title | Birthdate |
|---|---|---|
| Marcelino F. Sierra, Ph.D. | Asst. Attending Clinical Microbiologist | REDACTED |

Place of Birth

REDACTED

Sex

Male

## Education

| Institution | Degree | Year Conferred |
|---|---|---|
| City College of New York | B.S. (Biology) | |
| Wagner College, New York | M.S. (Bacteriology) | REDACTED |
| St. John's University, New York | Ph.D. (Microbiology-Biochemistry) | |

## Major Research Interest

Energy metabolism of cells in the normal situation and during impaired function.

## Publications

1. Sierra, M.F. and Tzagoloff, A. Properties of a subunit of yeast ATPase synthesized by mitochondrion. Fed. Proc. 31:463 Abstract 1972.

2. Tzagoloff, A., Akai, A., and Sierra, M. Assembly of mitochondrial membrane system VII. Synthesis and integration of F-1 subunits into the rutamycin-sensitive ATPase. Accepted for publication: J. Biol. Chem. (1973)

3. Tzagoloff, A., Meryl, R., and Sierra, M. Biosynthesis of mitochondrial enzymes. Accepted for publication: Biochem. Biophys. Acta. (1973)

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## Major Work Planned for Grant Period 7/1/73 to 6/30/74*

Investigative work during the ensuing grant year will include studies of the glucose metabolism and hydrolytic enzyme activity of basal and mobilized alveolar macrophages harvested from control and smoke-exposed animals under the same conditions of smoke-exposure used to assess phagocytosis and intracellular killing activity.** This information is desirable, since energy output and cell metabolism represent important potential links in the successful mobilization and subsequent antibacterial activity of alveolar macrophages. Indeed, alterations in hydrolytic enzyme activity may interfere with the primary immunological function of macrophages in pulmonary defense, namely, the destruction of inhaled microorganisms.

(1) Respiration studies: Experiments are planned which will permit determinations of the oxygen consumption and lactic acid content of alveolar macrophages incubated in basal medium and media supplemented with glucose plus serum with and without the presence of particles that induce phagocytosis. These studies will be performed with the following categories of macrophages: (a) basal macrophages harvested from control and smoke-exposed mice and (b) mobilized macrophages harvested from control and smoke-exposed mice immediately after exposure to aerosols of a phosphate buffer for 30 minutes.

Measurements of oxygen uptake will be determined in a Gilson respirometer using flasks with a 15 ml capacity containing monolayers of alveolar macrophages in a total liquid volume of 3.2 ml(1). The $CO_2$ will be absorbed by 0.2 ml of 20% KOH in the center well. In accordance with the protocol of each study, glucose (5.0 to 10 M) and polystrene spheres at a concentration of 2.0 to 2.5 mg/ml will be introduced via the side arm. After completion of the oxygen consumption measurements, the cells will be harvested and their lactic acid content determined by the method of Barker et al (2). For this purpose, the recovered macrophages will be washed in saline and cell extracts are to be prepared as described by Myrvik et al (3).

(2) Glucose metabolism studies: Experiments with specifically labelled glucose as substrate will be performed to evaluate the effect of cigarette smoke on the metabolism of glucose by alveolar macrophages. These studies will be done with the same categories of basal and mobilized macrophages used in the respiration studies.

The radioactive measurements will be done as described by Myrvik et al (3). Alveolar macrophages are harvested and placed in Erlenmeyer flasks containing medium 199 without serum or glucose. In separate experiments BCG and heat killed staphylococci will be added to all flasks except the control flasks. After incubation at 37°C in a shaker bath for 1, 2, 4, 6, and 18 hours, glucose 1-$C^{14}$ or glucose 6-$C^{14}$ is added to the flasks, and reincubated for 1 hour. The reaction is stopped with sulfuric acid and counts obtained with a liquid scintilation counter.

(3) Hydrolytic enzyme studies: The present grant proposal will include a study of the enzymatic activity and intracellular distribution of a specific group of hydrolytic enzymes in basal and mobilized macrophages harvested from control and smoke-exposed animals. The enzymes acid phosphatase, lysozyme, lipase, beta-glucuronidase and cathepsin are of immediate interest because their activity is increased in the BCG induced alveolar macrophage (3-6). To accomplish these aims, the enzymatic activity detectable in the cell free supernatant fraction and alveolar macrophage fraction of lung harvests will be assayed. Interest in both fractions of lung harvests resides in the fact that bronchial mucus contains several poorly defined substances(7)

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*The methods, procedures and conditions of smoke and aerosol exposure have been presented in detail in the original proposed project research plan of CTR Grant 547C (Jan. 18, 1971).

**Details of in vitro phagocytosis system has been presented in detail in Progress Report No. 1 for Grant #547-B covering the grant period 7/1/69 - 12/31/69.



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*including lysozyme* (8) that exert nonspecific bacteriostatic and bactericidal activity against gram positive and gram negative bacteria. In addition, alveolar macrophages by virtue of their high hydrolytic enzyme content, presence in large numbers and rapid turnover rate may contribute to the enzymatic activity found in mucus secretions.

3(a) Enzyme activity of lung harvests: A comparison will be made of the enzymatic activity and intracellular distribution of the above hydrolases in the following categories of macrophages: (a) basal macrophages harvested from control and smoke-exposed animals and (b) mobilized macrophages harvested from control and smoke-exposed animals immediately after exposure to aerosols of viable or heat-killed staphylococci. For this purpose, smoke-exposed and control animals will be exposed to the bacterial aerosols for 30 minutes. Enzyme determinations will be made with the lung harvests obtained from both groups of animals immediately after bacterial challenge and at hourly intervals over a 4 hour post-aerosol exposure period. The choice of laboratory animals (mice, guinea pigs, rabbits) to be used in all enzyme studies will be governed by the minimal yields necessary for adequate enzyme assay. The lung washing obtained from animals by the lavage technique will be separated into a cell-free supernatant fraction and cellular fraction containing alveolar macrophages by centrifugation at 4°C for 15 min at 10,000 rpm (8). The supernatant will be frozen at -60°C and stored until assay. The harvested alveolar macrophages will be washed twice in phosphate buffered saline (pH 7.2); quantitated by hemocytometer count; and viability assessed. Saline extracts of the washed cells are to be prepared for enzyme activity studies as outlined by Myrvik et al(3): disruption by alternate freezing and thawing for 5 consecutive cycles and removal of cellular debris by centrifugation at 2,500 rpm for 10 min at 4°C. The completeness of cell disruption will be audited by phase optics. In order to evaluate the contribution made by alveolar macrophages to the enzymatic activity detectable in the supernatant fraction, in vitro studies of the rate of release of the enzymes in question by pulmonary macrophages are planned. As outlined by Holzman et al (8) large numbers of alveolar macrophages will be suspended in tissue culture medium and samples of the cell population assayed for specific enzymatic activity after incubation at 37°C for various periods of time. In studies of the intracellular distribution of the hydrolases, alveolar macrophages will be suspended in a 0.25M sucrose solution, ruptured by homogenization and sedimented by centrifugation into four fractions (4). The nuclear fraction (N) will be sedimented by centrifugation at 250 X G; the heavy granule fraction (HG) at 5000 X G for 15 min; and the light granular fraction (LG) and supernatant fraction (S) by centrifugation at 25,000 X G. Each fraction will be submitted to 5 cycles of freezing and thawing and clarified by centrifugation at 2500 X G for 20 min and subjected to enzyme assay.

3(b) Antibacterial activity of supernatant fraction of lung harvests: In order to study the antibacterial properties of pulmonary secretions, the supernatant fraction of lung harvests obtained by the lavage technique will be inoculated with fixed numbers of viable bacteria, incubated at 37°C for various periods of time and the viability of the bacteria assessed by colony counts obtained by a standard agar pour plate technique. The microorganisms to be used as a bacterial challenge include S.aureus, S.albus, other gram positive bacteria and several gram negative bacteria. These studies will be performed with the supernatant fraction of lung harvests obtained from the following categories of animals: (a) basal macrophages harvested from control and smoke-exposed animals and (b) mobilized macrophages harvested from control and smoke-exposed animals challenged with aerosols of carbon particles or heat-killed staphylococci.



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For the purpose of enzyme analysis, lysozyme will be quantitated by using suspensions of Micrococcus leisodyktious as substrate. Tests will be standardized with known amounts of crystalline egg white lysozyme and results expressed as egg white lysozyme equivalents. Acid phosphatase will be measured by the procedure of Hofstee (9) using O-carboxyphenyl phosphate as substrate. An increase in absorbance of 0.001 optical density units/min under standard conditions will be taken to represent a unit of activity. Beta-glucuronidase will be assayed by the procedure of Fishman et al (10) using phenolphthalein mono-Betaglucuronide as substrate with reactions carried out in 0.1M acetate buffer at pH 4.5 and 38°C. One unit of glucuronidase is the activity resulting in the liberation of 1 ug/hr phenolphtalein. Lipase activity will be determined as outlined by Cohn et al (5) in which naphthol laurate serves as substrate and the increase in activity caused by sodium taurocholate is taken to represent minimal lipase activity. The results are to be expressed as micromoles of naphthol liberated per hour. Cathepsin will be assayed employing a 2% solution of denatured hemoglobin as a substrate, as described by Anson (11) and protein digestion estimated with a spectrophotometer by absorption at 280 mu (4). A unit of activity being defined as an increase in optical density produced by 0.001 meq of tyrosine.

## References


1. Oren, R., Farnham, A. E., Saito, K., Milofsky, E., and Karnovsky, M. L.: Metabolic patterns in three types of phagocytizing cells, J. Cell, Biol. 17: 487, 1963.

2. Barker, S. B. and Summerson, W. H.: The colorimetric determination of lactic acid in biological material, J. Biol. Chem. 138: 535, 1941.

3. Myrvik, Q. N. and Evans, D. G.: Metabolic and immunological activities of alveolar macrophages, Environ. Health 14: 92, 1967.

4. Meise, E. R., Myrvik, Q. N. and Leake, E. S.: Effects of Bacillus Calmette Guerin on the levels of acid phosphatase, lysozyme and cathepsin in rabbit alveolar macrophages, J. Immun. 95: 125, 1965.

5. Cohn, Z. A. and Wiener, E.: The particulate hydrolases of macrophages: I. Comparative enzymology, isolation and properties, J. Exp. Med. 118: 991, 1963.

6. Cohn, Z. A., and Wiener, E.: The particulate hydrolases of macrophages. II. Biochemical and morphological responses to particle ingestion, J. Exp. Med. 118: 1009, 1963.

7. Miller, T. E. and Watson, D. W.: Innate immunity, Med. Clin. N. Am. 49: 1489, 1968.

8. Holzman, R. A., Gardner, D. E. and Coffin, D. L.: In vivo inactivation of lysozyme by ozone, J. Bacterial 96: 1562, 1968.

9. Hofstee, B. H. J.: Direct and continuous spectrophotometric assay of phosphomonoesterases, Arch. Biochem. 51: 139-146, 1954.

10. Fishman, W. H., Springer, B. and Brunetti, R.: Application of improved glucuronidase assay method to study of human blood B-glucuronidase, J. Biol. Chem. 173: 449, 1948.

11. Anson, M. L.: The estimation of pepsin, trypsin, papain, and cathepsin with hemoglobin, J. Gen. Physiol. 22: 79, 1938.




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13. Budget for the coming year:

| | hrs/week % time | Amount |
|---|---|---|
| A. Salaries (give names or state "to be recruited") Professional (give % time of investigator(s) even if no salary requested) (See Page 3A) | | |
| Joseph J. Guarneri, Ph.D. Principal Investigator | 20 hrs/week | REDACTED |
| Marcelino Sierra, Ph.D. | 10 hrs/week | |
| Technical | | |
| Michael Veit, M.S. | 100%- 35 hrs/wk. Fringe benefits | |
| Research Assistant | 30% - 12 hrs/wk. Fringe benefits | |
| Sub-Total for A | | |
| B. Consumable supplies (by major categories) (See page 3A for itemization) | | |
| Sub-Total for B | | $4,650 |
| C. Other expenses (itemize) Travel $350 Publications 150 | | |
| Sub-Total for C | | $ 500 |
| Running Total of A + B + C | | $18,577 |
| D. Permanent equipment (itemize) (see Page 3A) Gilson Differential Respirometer with 8 volumometers and mobile base (Model G 14) | | |
| Sub-Total for D | | 1,580 |
| E. Indirect costs (15% of A+B+C) | | 2,787 |
| Total | | $22,94[illegible] |

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## Justification and Explanation of Budget

Budget Category: (A) Salary: The increase over 1972 in the salary category of $972 reflects the anticipated increment in salaries and accompanying fringe benefits during the grant period of 7/1/73 to 6/30/74.

Budget Category: (B) Itemization of Consumable Supplies:

| | |
|---|---|
| Mice Swiss Webster, Caesarian Delivered, including feed | $ 1,000 |
| Rabbits | 1,100 |
| Cigarettes for smoke studies | 800 |
| Petri dishes, plastic disposable | 200 |
| Tissue culture glassware, media and serum | 600 |
| Bacteriology media and defibrinated blood | 200 |
| Isotapes, uniformly labelled glucose -1-$C^{14}$ and glucose 6-$C^{14}$ | 300 |
| General Lab, glassware and reagents | 150 |
| Reagents and curvettesfor enzyme studies and flasks and accessories for respiration studies | 300 |
| | $4,650 |

Budget Category: (D) Permanent Equipment

The Gibson Differential Respirometer is needed for the respiration and isotopes studies proposed in the original grant request and the current grant renewal. The respirometer was not included in the projected equipment needs of the original grant request made in 1971 because it was then available at St.Vincent Hospital. At the present institution this instrumentation is not available to my research laboratory.

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14. Other sources of financial support.

List financial support from all sources, including own institution, for this and related research projects.

CURRENTLY ACTIVE

| Title of Project | Source (give grant numbers) | Amount | Inclusive Dates |
|---|---|---|---|
| (1) The Influence of Extended Exposure to Cigarette Smoke on Pulmonary Resistance to Infection as Related to Alveolar Macrophage and Mucociliary Function. | The Council for Tobacco Research-USA. Grant Nos. 547 and 547C | $40,208 | 7/1/71 to 6/30/73 |

PENDING OR PLANNED

| Title of Project | Source (give grant numbers) | Amount | Inclusive Dates |
|---|---|---|---|
| (1) The Influence of Extended Exposure to Cigarette Smoke on Pulmonary Resistance to Infection as Related to Alveolar Macrophage and Mucociliary Function. | The Council for Tobacco Research-USA. (Current renewal application) | $22,944 | 7/1/73 to 6/30/74 |
| (2) The Influence of Bacterial Species on the In Vitro Antibacterial Activity of Alveolar Macrophage. | Long Island Jewish-Hillside Medical Center (to be submitted 7/1/73) | 6,000 | 7/1/73 to 6/30/74 |
| (3) The Mechanisms of Pulmonary Defense Against Infection. | Dep't. Health, Education and Welfare, Public Health Services (To be submitted 6/1/73) | 180,000 | 1/1/74 to 12/31/77 |

It is understood that the investigator and institutional officers in applying for a grant have read and accept the Council's "Statement of Policy Containing Conditions and Terms Under Which Project Grants Are Made."

Principal investigator

Typed Name Joseph J. Guarneri, Ph.D.

Signature [redacted] Date 1/24/73

Telephone [redacted]
Area Code Number Extension

Checks payable to

Long Island Jewish-Hillside Medical Center

Mailing address for check: Mrs. Eva Meyers, Grant Mgr.
Long Island Jewish-Hillside Medical Center
New Hyde Park, New York 11040

Responsible officer of institution

Typed Name Robert K. Match, M.D.

Title Executive Vice President & Director

Signature [redacted] Date

Telephone [redacted]
Area Code Number Extension

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